February 15, 2006

Edwin L. Mongan, III
Manager, Environmental Stewardship
E.I. du Pont de Nemours & Company, Inc.
1007 Market Street
DuPont 6082
Wilmington, DE 19898

Dear Mr. Mongan:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for Aminoalkylnitriles Category posted on the ChemRTK HPV Challenge Program Web site on September 9, 2004. I commend E.I. du Pont de Nemours & Company, Inc. for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that DuPont advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission. Please send any electronic revisions or comments to the following e-mail addresses: oppt.ncic@epa.gov and chem.rtk@epa.gov.

If you have any questions about this response, please contact Mark Townsend, Chief of the HPV Chemicals Branch, at 202-564-8617. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

/s/

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: W. Penberthy
J. Willis

EPA Comments on Chemical RTK HPV Challenge Submission:
Aminoalkylnitriles

Summary of EPA Comments

The sponsor, E.I. du Pont de Nemours and Company, submitted a test plan and robust summaries to EPA for the Aminoalkylnitriles, dated April 7, 2004. EPA posted the submission on the ChemRTK HPV Challenge Web site on September 9, 2004. The submission consists of 2-amino-2-methylpropanenitrile (CAS No. 19355-69-2) and 2-amino-2-methylbutanenitrile (CAS No. 4475-95-0). Data for 2-amino-2,3-dimethylbutanenitrile (CAS No. 13893-53-3) are included in the submission as supporting information.

EPA has reviewed this submission and has reached the following conclusions:

1. Analog Justification. Both sponsored chemicals and the analog are said to break down in the same way in water. No information about reaction rates, nor the importance of the reaction products in the overall toxicity of these substances, is presented. The submitter needs to provide this information to clarify how to apply toxicity information on the analog to the sponsored chemicals.

2. Category Justification. The similarities in structure, reactivity, uses, predicted physicochemical properties, and toxicity to aquatic species support grouping the 2-aminoalkylnitriles.

3. Physicochemical Properties. The submitter needs to provide measured melting point and water solubility data for the sponsored chemicals. For boiling point, the submitter needs to provide decomposition temperatures. The submitter needs to indicate in the robust summaries whether the vapor pressure data are measured or estimated and, if estimated, provide measured values for both chemicals.

4. Environmental Fate. The submitter needs to provide experimental data for stability in water. The submitter also needs to provide measured ready biodegradation data for both sponsored chemicals, taking into account the rates of chemical reaction in water.

5. Health Effects. (a) Adequate data are available for acute toxicity. (b) EPA reserves judgement on the adequacy of the gene mutation data pending submission of additional information. (c) EPA agrees with the submitter's proposal to conduct *in vitro* chromosomal aberration and developmental toxicity testing on 2-amino-2-methylpropanenitrile and recommends a combined repeated-dose/reproductive/developmental toxicity screening test instead of the proposed developmental toxicity test. (d) The submitter needs to provide additional information to satisfy the requirements for classification of aminoalkylnitriles as closed system intermediates (CSIs).

6. Ecological Effects. The submitted data are adequate for all ecological endpoints for the purposes of the HPV Challenge Program. The submitter needs to provide data elements missiing from the robust summaries.

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

EPA Comments on the Aminoalkylnitriles Challenge Submission

Category Definition

The submission contains two sponsored chemicals: 2-amino-2-methylbutanenitrile and 2-amino-2-methyl-propanenitrile. Data for a non-sponsored compound, 2-amino-2,3-dimethylbutanenitrile, are included to support the grouping.

Analog Justification

Although the analog is cited in the test plan as the “next higher homolog”, the analogy is imperfect because it is not a classic straight-chain homolog but is branched, introducing steric crowding adjacent to the reactive site (as noted in the test plan discussion of biodegradation). Nonetheless, the analogy is potentially reasonable because of the postulated instability of all three molecules.

These aminoalkylnitriles are said to break down in water to cyanide, the corresponding ketone, and ammonia/ammonium ion. The language used is vague–“likely to be unstable in water”, “show a tendency to disproportionate”, “may disproportionate”, etc. Apart from a comment in one robust summary about increased reactivity at higher pH, no information about individual or relative reaction rates, nor the importance of the reaction products in the overall toxicity of these substances, is presented in the test plan. This information is important because, if cyanide is the most important determinant of toxicity and the disproportionation rate of the analog differs significantly from the sponsored chemicals, then it will be unclear how to apply toxicity information developed for the analog to sponsored substances. Therefore, the submitter needs to provide this information.

Category Justification

The submitter bases its grouping of the 2-aminoalkylnitriles on the similarities in molecular structure, reactivity, uses, predicted physicochemical properties, environmental toxicity and mammalian acute toxicity. The submitter states that the two sponsored compounds are nearest structural homologs and should have similar physicochemical, environmental, and toxicological properties. The supporting compound has one additional carbon and should thus have properties similar to the sponsored compounds.

Among the physicochemical properties, the estimated values given for melting point and partition coefficient for the sponsored compounds are similar. Both chemicals decompose before boiling. The vapor pressure values for both chemicals are also similar, though it is not clear if these data are calculated or measured. The estimated water solubility values differ. However, as a rule, similar estimated properties do not provide additional support for grouping closely structurally similar compounds because the estimated values simply reflect the already apparent structural similarities.

Likewise, estimated environmental fate data are of little use in further supporting the grouping of the 2-aminoalkylnitriles.

Acute oral LD_{50} data are similar among the three 2-aminoalkylnitriles. Data for 2-amino-2-methylpropane-nitrile and 2-amino-2,3-dimethylbutanenitrile from acute inhalation and dermal studies also indicate similar acute toxicities. Mutagenicity data are provided only for the supporting compound, 2-amino-2,3-dimethyl-butanenitrile, and no clastogenicity data are available for any of the three. Limited data on repeated-dose toxicity on the sponsored chemicals cannot support the grouping because of the use of different exposure routes and durations. Data are not available for the reproductive and developmental endpoints. Thus, a lack of adequate or comparable data for genetic, repeated-dose, reproductive, and developmental toxicities precludes a mammalian toxicity comparison of the 2-aminoalkylnitriles. However, similar repeated-dose, genetic, and reproductive/developmental toxicities are possible, given the likelihood that the 2-aminoalkylnitriles are metabolized *in vivo* by a common pathway.

Measured data provided for the acute fish and invertebrate toxicity endpoints support the grouping of the 2-aminoalkylnitriles. Although measured algal toxicity data are available only for the analog 2-amino-2,3-dimethybutanenitrile, the measured fish and invertebrate EC_{50} values suggest that the three compounds would have similar aquatic plant toxicities as well.

Overall, despite the above reservations, EPA considers that the structural similarities, reactivities, and ecotoxicological properties of the 2-aminoalkylnitriles provide a reasonable basis for initially grouping the sponsored compounds.

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility)

The submitted data for partition coefficient are adequate for the purposes of the HPV Challenge Program.

Melting point. The melting point value provided by the submitter for 2-amino-2-methyl-butanenitrile is not adequate because estimated melting point values above 0° C are not acceptable for the purposes of the HPV Challenge Program. The submitter needs to provide a measured melting point value for this chemical following OECD TG 102. Because the estimated melting point for 2-amino-2-methylpropane-nitrile is only slightly below 0° C, and because melting points calculated by EPIWIN often depart from measured values, EPA strongly encourages the submitter to provide measured data for this chemical also.

Boiling point. The submitter stated that all three chemicals decompose with heat but did not provide decomposition temperatures. The submitter needs to include this information in the robust summaries.

Vapor pressure. In the robust summaries, the submitter provided vapor pressure values obtained from company MSDSs (30 mm Hg at 66 °C, 14 mm Hg at 68 °C). In the test plan (page 3) the submitter states that these two values are measured; the submitter needs to verify this information and include it, with adequate details or citations, in the robust summaries. If these values are estimated, they are not adequate for the purposes of the HPV Challenge Program and the submitter needs to provide measured data for both chemicals following OECD TG 104.

Water solubility. The estimated water solubility values provided by the submitter for both substances are not adequate because estimated water solubility values above 1μg/L (1ppb) are not adequate under the HPV Challenge Program. Without more quantitative information about the stability of the chemicals in water, the practicality of testing them for water solubility is unknown. Given the available information, the submitter needs to provide measured water solubility values for the sponsored chemicals following OECD TG 105.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

The submitted data for photodegradation and fugacity are adequate for the purposes of the HPV Challenge Program.

Stability in water. The submitter needs to provide measured stability-in-water data, including reaction rates, for both chemicals following OECD TG 111.

Biodegradation. The submitter needs to provide measured ready biodegradation data for both chemicals following OECD TG 301, taking into account the rate of transformation. If these chemicals react rapidly, then the submitter needs to provide measured ready biodegradation data for the reaction products.

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

Adequate data are available for acute toxicity. EPA agrees with the submitter's proposal to conduct an *in vitro* chromosomal aberration test on 2-amino-2-methylpropanenitrile following OECD TG 473.

Genetic toxicity (gene mutations). Although the data for the proposed analog in a reverse mutation assay in several strains of *Salmonella typhimurium* with and without metabolic activation appeared adequate in EPA's previous review (EPA Comments on 2-amino-2,3-dimethylbutanenitrile, http://www.epa.gov/oppt/chemrtk/2amindi/c13131ct.htm), the negative results as currently presented do not permit reliable extrapolation to the sponsored chemicals. No information was provided on test substance purity or whether the chemical was cytotoxic. Additional missing study details include incubation conditions (e.g., temperature and duration), criteria for a positive response, mean number of revertant colonies per plate for treated and control cultures, whether or not positive and negative controls gave the appropriate response, and statistical methods. EPA reserves judgement on the adequacy of the gene mutation data pending submission of the missing information. The purity/composition of the tested substance is particularly important in determining whether the data on 2-amino-2,3-dimethylbutanenitrile would adequately represent the toxicity of the sponsored chemicals, given the stated instability of these chemicals.

Repeated-dose and reproductive toxicity. The submitter's claim of a CSI exemption from testing these endpoints is addressed later in this section.

The submitted repeated-dose data are inadequate. The 14-day repeated-dose inhalation toxicity study of 2-amino-2-methylpropanenitrile in male rats and a 28-day repeated-dose dermal neurotoxicity study of 2-amino-2,3-dimethylbutanenitrile in male and female rats are inadequate for several reasons: (1) the 14-day study does not meet the 28-day minimum duration requirements for the repeated-dose toxicity endpoint, did not test animals of both sexes, and did not produce any adverse effects at the highest concentration tested; (2) the 28-day study was conducted primarily to examine effects on the nervous system, rather than systemic effects, and the highest tested concentration was much lower than the OECD guideline requires; (3) without information on the relative rates of disproportionation in water and the importance of the reaction products to the overall toxicity of the category, it is unclear whether the data generated on 2-amino-2,3-dimethylbutanenitrile can represent the toxicity of the category. Testing is needed to address repeated-dose toxicity.

No data were submitted for the reproductive toxicity endpoint.

Although the submitter has satisfied many of the criteria for claiming the aminoalkylnitriles as CSI, additional information is needed to qualify for reduced testing of these chemicals.

The Guidance for Testing Closed System Intermediates for the Challenge Program at http://www.epa.gov/chemrtk/guidocs.htm allows for a reduced testing proposal provided certain criteria are met. The information required to judge a "closed system intermediate" claim must address the following:

I. Site information.
- A. Number of sites.
- B. Basis for "closed process" conclusion at each site.
  - 1) Process description.
  - 2) Monitoring data showing no detection.
  - 3) In the absence of monitoring data, the basis for believing that releases do not occur.
- C. Data on "presence in distributed products."

II. Information on transport (mode, volume, controls, etc.)

III. A data search showing that the chemical is not present in other endproducts.

The submitter has generally addressed the criteria described above except for the number of sites. The IUR information submitted for the years 1998 and 2002 indicated that the subject chemicals were manufactured by another company in 2002, and EPA does not have information to document that the chemicals were manufactured and used in closed systems. In addition, there is no information on the disposition of vapors from sampling hoods and from purging of lines used to load the chemicals. The submitter needs to indicate if vapors leaving the laboratory hood are directed to a flare or are otherwise controlled. The submitter also needs to indicate that these chemicals are not present in any products produced using these chemical substances and that there are no releases to the environment in wastes or cleaning solution. EPA therefore reserves judgment on whether aminoalkylnitriles meet the criteria for CSIs pending the submission of additional information. *Conducting a combined repeated-dose/ reproductive/developmental toxicity screening test (OECD TG 422) as recommended in the following section would obviate the need to sustain a CSI claim.*

Developmental toxicity. The submitter plans to conduct a developmental toxicity test (OECD TG 414). However, HPV Challenge Program guidance recommends that a combined repeated-dose/reproductive/ developmental toxicity screening test (OECD TG 422) be conducted instead.

Ecological Effects (fish, invertebrates, and algae)

Collectively, the data are adequate for all ecological endpoints. The submitter needs to provide missing data elements in the robust summaries.

Specific Comments on the Robust Summaries

Ecological Effects

Fish. Missing study details include: hardness and total organic carbon of the dilution water, temperature, number of fish per test vessel, fish loading, mean fish length/weight, and 95% confidence limits.

Invertebrates. The robust summary for daphnia 48-hour static toxicity of 2-amino-2-methylpropane-nitrile is missing the following details: hardness and total organic carbon of the dilution water, temperature, pH, dissolved oxygen, age of the daphnids, and 95% confidence limits. The robust summary for daphnia 48-hour static toxicity of 2-amino-2,3-dimethylbutane is missing test substance purity, hardness and total organic carbon of the dilution water, and age of the daphnids. Dissolved oxygen and pH values were measured during the study, but were not reported in the summary.

Algae. Missing study details for 2-amino-2,3-dimethylbutanenitrile include test substance purity, lighting conditions, pH, and whether the EC_{50} was based on measured or nominal concentrations. References to the statistical determination of an LC_{50} should be revised to reflect determination of an EC_{50}.

Followup Activity

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.